

## Mating Behaviour of an Undescribed Species of *Coccophagus*, Near *C. gurneyi* (Hymenoptera: Aphelinidae)

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**Abstract.**—We detail the mating behaviour of an undescribed species of *Coccophagus* that resembles *C. gurneyi* Compere anatomically, and which is also an Australian parasitoid of mealybugs. The pattern of the male-female interaction was complex and protracted, which is typical of many species in the genus. All three principal behavioural categories, namely pre-coital courtship, coitus and post-coital mount behaviour, were expressed. The post-coital aspect was longest, which is common in the genus and family. Wing flicking during coitus and head nodding movements during post-coital behaviour were two distinct displays of the males that have not so far been reported in other *Coccophagus* species. Attraction of the males to virgin females in the field is demonstrated and is the first direct evidence of long distance pheromonal attraction of mates in the genus *Coccophagus*, and which has been documented only occasionally in other hymenopterous parasitoids.

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### INTRODUCTION

Cryptic species complexes exist in the genus *Coccophagus* Westwood and the intergradation of structural traits complicates the resolution of species limits (Compere 1931; Annecke and Insley 1974; Walter 1993). Colour patterns vary intraspecifically within some species groups in the genus and cannot be used alone in classification (Annecke and Insley 1974; Walter 1993). In contrast, observations on mating behaviour have sometimes provided a good taxonomic tool for accurate diagnosis of the *Coccophagus* species that have elaborate and protracted behaviour associated with mating (Battaglia *et al.* 1988; Walter 1993).

Chalcidoid mating behaviour is typically divided into three phases, the pre-coital phase, coitus and post-coital mount behaviour (Gordh and DeBach 1978). This division, though arbitrary, has been useful in conceptualising the mating behaviour of two closely-related *Coccophagus* species, *C. bartletti* Annecke and Insley and *C. lutes-*

*cens* Compere (Walter 1993). So far, the mating behaviour of only eight *Coccophagus* species has been described in any detail (Table 1). The sequences of many others (e.g., *C. capensis* Compere and *C. ochraceus* Howard), although reported, are still incompletely known. Long-distance pheromonal attraction of males by virgin females of *Coccophagus* species may initiate sexual communication (Walter 1993), but controlled tests have never been conducted.

Here we report the mating behaviour and role of sex pheromones in an undescribed species of *Coccophagus* that is anatomically similar to *C. gurneyi* Compere, but which differs in head colour, ratio of ovipositor length to middle tibial length (G.L. Prinsloo, South African National Collection of Insects, *in litt.*, 15. vii. 1994), and in aspects of its host relationships (Abeeluck 1995). Voucher specimens have been deposited in the Queensland Museum, Brisbane.

The male eggs of *C. gurneyi* reputedly follow one of two quite different devel-

Table 1. Summary of published analyses of mating behaviour of *Coccophagus* species. Mating is divided into precoital courtship, coitus and postcoital courtship, and the duration (seconds) of each is presented. Signals and appendages used in precoital and postcoital display are differentiated. Females of those species with an asterisk are known to be monandrous.

Species	Duration (secs)			Signals and appendages used				Author(s)
	Precoital courtship	Coitus	Postcoital courtship	Pheromones	Wings	Antennae	Legs	
* <i>C. atratus</i> Compere	2-10	0	0	Pre	?	Pre	?	Donaldson et al. 1986
* <i>C. bartletti</i> Annecke & Insley	15	4	90	Pre	Post	Pre	?	Walter 1993
<i>C. capensis</i> Compere	<1	?	?	?	?	?	?	Flanders 1937
* <i>C. lutescens</i> Compere	5	3	48	Pre	Post	Pre	Post	Walter 1993
<i>C. matsuyamensis</i> Ishii	7	4	22	?	Post	Pre	Post	Battaglia et al. 1988
<i>C. obscurus</i> Westwood	6	15	0	?	?	Pre	?	Battaglia et al. 1988
<i>C. ochraceus</i> Howard	Few secs	Few secs	0	—	?	?	?	Cendaña 1937
<i>C. varius</i> Silvestri	Reported to be the same as that for <i>C. matsuyamensis</i> (see above) Battaglia et al. 1988							

opmental pathways, depending on whether the mealybug that will host the male egg already contains a parasitoid larva or pupa at the time of oviposition (Flanders 1964). Conceivably, however, Flanders made observations on wasps from more than one unrecognised sibling species whose males differ from each other in their host relationships (Walter 1983). Our general aim is, therefore, to help resolve the question of species limits and host relationships in the *gurneyi* "group" of *Coccophagus* species.

## MATERIALS AND METHODS

Females of *Coccophagus* sp. nr *gurneyi* were collected from *Phenacoccus parvus* Morrison on *Lantana montevidensis* (Sprengel) Briq. on the St Lucia Campus of The University of Queensland. To determine whether long distance pheromonal attraction is involved in the sexual communication of this species, two cages with 15 virgin females in each and two with 15 mated females in each (all females were one day old) and one control cage without

females were placed one meter apart, at ground level, in *L. montevidensis* patches infested with *P. parvus*. The cages were observed continuously for 60 minutes, between 1000h to 1100h, and all small wasps that landed on them were captured. The position of the cages was then interchanged for a further 60 minutes of observation. The procedure was followed for three consecutive days. Each captured insect was stored alone in a vial with honey. All vials were kept cool in an insulated box for transport to the laboratory, where each male *Coccophagus* individual that had been captured was placed with a virgin *Coccophagus* female to check if they would mate.

For detailed behavioural observations, each virgin female ( $n = 17$ ) was released into a vial (35 mm  $\times$  10 mm diameter) containing a male of unknown age and mating status. (The usual hosts of the males of this species of *Coccophagus* are not known, so laboratory reared individuals were not available.) Observations were made at  $25 \pm 2^\circ\text{C}$  under a dissecting

microscope (40× magnification) with a heat-filtered tungsten light and were recorded on video tape. Following copulation, the male was discarded. To confirm successful insemination, each female was killed in 70% alcohol and transferred to a drop of 1% saline. The spermatheca was detached, covered with a coverslip in a drop of 1% saline, and examined under phase contrast for the presence of sperm. The spermathecal capsule was rated 100% full when sperm occupied the entire cavity within the capsule (Abeeluck 1995).

Females were also tested to establish whether, after first insemination, they remained receptive to males that attempted to mate. One-day old females ( $n = 11$ ) were each exposed to a single male in a vial. After mating, the females were isolated with a drop of undiluted honey and the males were discarded. After 24 hours each female was again exposed to a male and observed for 20 minutes, a procedure that was followed for seven days. Most mated females (60%) died on the eighth day, when observations were stopped.

To determine whether males could mate with more than one female, single males ( $n = 7$ ) were each placed in a vial (35 mm × 10 mm) with three virgin females. When each male had mated with all three females, he was isolated and the females were dissected to assess the sperm content of their spermathecal capsule. A further set of three virgin females was exposed to each of the males 30 minutes later; the females were dealt with as before.

## RESULTS

Cages with virgin females attracted males, whereas those with mated females and empty cages did not (Table 2), even when the position of the cages was changed. The mean time between initial exposure of cages and the arrival of the first male was 10 ( $\pm 2.5$ ) minutes ( $n = 12$ ). Males flew in a zig-zag course, 10–20 cm above the cage, for 25 ( $\pm 3.4$ ) seconds before landing on the cage ( $n = 12$ ). Once on

Table 2. Catches of *Coccophagus* sp. nr *gurneyi* males at cages that held virgin or mated females or that had no wasps (controls) ( $n = 2$  cages for each treatment).

Day	Trap type	Number of males caught	
		0–60 minutes	60–120 minutes
1.	Virgin female*	18 & 21	11 & 10
2.		19 & 16	10 & 12
3.		17 & 19	9 & 11
	Mean ( $\pm$ S.E.)	18.3 ( $\pm$ 0.7)	10.5 ( $\pm$ 0.4)
1.	Mated female	0 & 0	0 & 0
2.		0 & 0	0 & 0
3.		0 & 0	0 & 0
1.	Control cages	0 & 0	0 & 0
2.		0 & 0	0 & 0
3.		0 & 0	0 & 0

\* Catches were not significantly different between replicates and among days ( $P > 0.05$ , Chi Square Test; Statview 1992), so replicates have been pooled. Catches made during the first period of exposure were significantly higher than those made during the second exposure (Wilcoxon Signed Ranks Test,  $P = 0.028$ , Statview 1992).

the cage, males walked around, antennating the substrate.

All field-collected males that were tested mated readily with virgin females. Successful insemination was confirmed by all mated females having their spermathecal capsules 100% full ( $n = 57$ ).

Mating behaviour followed a consistent pattern ( $n = 17$ ) made up of several behavioural categories and is represented diagrammatically in Fig. 1. The precoital phase comprised five categories, but was relatively brief overall. Postcoital mount behaviour was more protracted than the precoital phase, but was not as diverse behaviourally.

## Precoital Courtship

Following introduction into a vial, males and females ran around, apparently at random. The time until the male was first stimulated by the presence of the virgin female, characterised by his faster pace and rapid upward and downward questing movements of his antennae, was 49.8 ( $\pm 0.7$ ) seconds. He then approached the

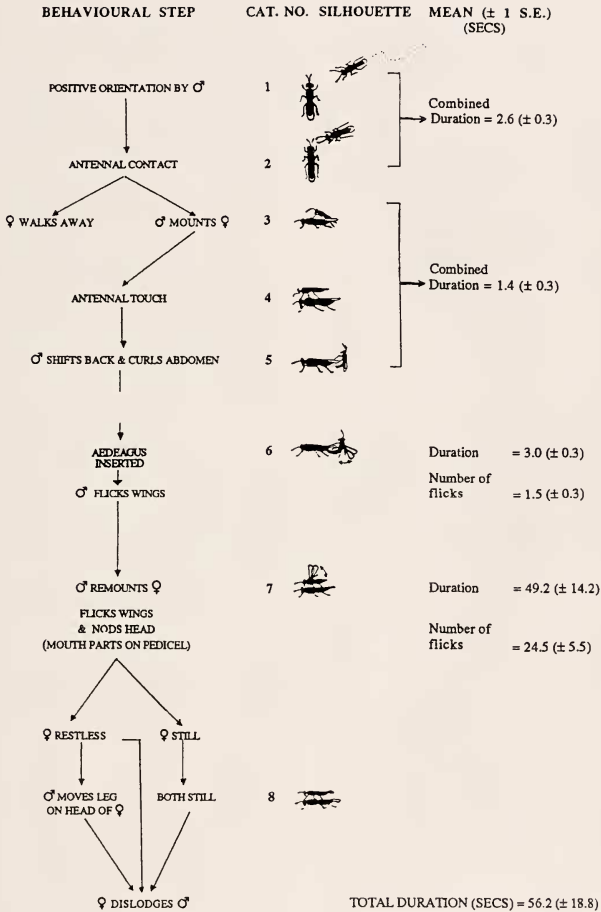


Fig. 1. Diagrammatic representation of the mating behaviour of *Coccophagus* sp. nr *gurneyi* (n=17).

female in a zig-zag pattern (category 1; Fig. 1). The male touched the female's antennae, either from the front or from the side, with his own antennae and then briefly touched her dorsal thorax with them (category 2). Unreceptive females walked away after initial contact with a male. Rejected males continued walking as they had when first introduced into the vial.

Receptive females stood still and allowed the male to mount her (category 3). Once on the female, the male moved forward to her head and touched her antennae with his own. Recognition was stimulated with a single touch and the male then moved back and curled his abdomen (category 5). Behavioural categories 1 to 5 were brief, their combined duration being only about four seconds on average (Figure 1).

### Coital Phase

The copulatory period was short ( $3.0 \pm 0.3$  seconds). The male inserted his aedeagus into the female's gonopore and at the same time flicked his wings ( $1.5 \pm 0.3$  flicks), but never more than four times. In three of 17 observations, males did not flick their wings. During coitus, the female remained quiescent with her antennae held in a geniculate posture and she maintained this copulatory position even when the male did not succeed in inserting his aedeagus at the first attempt. She allowed subsequent attempts to achieve intromission.

### Postcoital Mount Behaviour

After insemination, all males remounted the dorsum of their partner to stand on her. He placed his forelegs on her vertex and, with rhythmic head movements, moved his mouthparts along the antennal pedicel of the female. Simultaneously he flicked his wings, as detailed in Fig. 1. His head movements were not quantified but he continued with them until the female dislodged him. The period of postcoital mount was comparatively long (Fig. 1).

Throughout, the male flicked his wings and moved his head, as described above, in protracted or momentary bouts. Males also stood still for short periods.

In most observations, postcoital mount was terminated by the female. She either twisted her head, groomed her eyes with her forelegs, kicked the male's hind legs with hers or ran, which shook the male from her back. In response to each attempt to dislodge him, the male changed the position of his fore- and hind legs. Movements of his fore legs were thus conspicuous and gave the impression that the male either searched for a better hold for them or tried to "pacify" the female when she attempted to dislodge him. Both the female and male, when separated, walked away and preened. Females, once inseminated, were unreceptive if the male attempted to copulate a second time.

In the laboratory, males and females copulated at any time of the day and even at night, but this was not quantified. All males ( $n = 7$ ) mated with the two sets of three virgin females (all of which had spermathecal capsules 100% full of sperm). Females, after their first mating, were no longer receptive to males that attempted to mate, at least not for seven days ( $n = 11$ ). The mated females either flew or ran to elude an approaching male.

### DISCUSSION

Males of *Coccophagus* sp. nr *gurneyi* are polygynous, whereas the females are monandrous. Use of field-collected males for the observations reported is therefore unlikely to have influenced the recorded behaviour significantly.

Pheromones undoubtedly serve as distance attractants because males are attracted to caged virgins exposed in the field (Table 2). When males are close to females ( $< 35$  mm), the zig-zag pattern of the male's approach indicates that olfaction is used for close range mate attraction and location, as suggested also for other *Coccophagus* species (Donaldson *et al.* 1988; Walter

1993). Whether the same pheromone operates for both close-up and distance attraction is not certain, but close range (about 1.5 cm) communication between the sexes by means of cuticular volatiles has been recorded in the braconid *Diachasma mimorpha kraussii* (Fullaway) (Rungtjawanich 1994). Distance attraction of mates has been little studied in parasitic wasps (Powell and King 1984; Godfray 1994), and our demonstration of this phenomenon is apparently the first for any species of *Coccophagus*. In lepidopterous species the long-distance upwind orientation behaviour in responding insects may be elicited by one pheromone component whereas another component may evoke close-range responses (Roelofs *et al.* 1977). Visual orientation may also be involved when male moths, and perhaps parasitoids, get close to calling females.

Male antennation, either on the female's antennae (categories 2 and 4) or on her body (category 2), possibly indicates chemotactile mediated communication by means of cuticular compounds. The males of *Coccophagus* sp. nr *gurneyi* flick their wings during coitus, which is the first record for the genus. Its function has yet to be determined, but it is not necessary for successful insemination in species of *Aphytis* (Rao and DeBach 1969).

Males spend comparatively longer in postcoital mount than in the precoital or coitus phases. Protracted postcoital mounting has been described in six species of *Coccophagus*. In none of these do males touch the female's antennae with his mouthparts, as recorded for *C. sp.* nr *gurneyi*. In yet other species, postcoital mounting never occurs (e.g., *C. atratus* (Donaldson *et al.* 1986); *C. hemera* (Walker) (Zinna 1961); *C. obscurus* (Battaglia *et al.* 1988). The possible functions of postcoital mount behaviour in aphelinids have been discussed by Kajita (1986) and Walter (1993).

The possibility that *C. gurneyi* comprises a complex of species has been expressed

by Walter (1983), based on Flanders' (1964) description of the "dual ontogeny" of *C. gurneyi* males. That *Coccophagus* sp. nr *gurneyi* goes through a series of postures and behaviours that are complex, stereotyped and easily visible suggests that observations on mating behaviour should help to resolve possible species problems in the taxon *C. gurneyi*. However, the mating behaviour of *C. gurneyi* remains undescribed.

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